Elevated atmospheric CO$_2$ affects structure of a model regenerating longleaf pine community

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Abstract

Differences in plant morphology, physiology, life form, and symbiotic relationships can generate differences in species responses to CO$_2$-enrichment, which can alter competitive interactions, thus affecting community structure and function. Here, we present data from a two-year study, examining the species and community responses to elevated [CO$_2$] of a model regenerating longleaf pine community. The model community was constructed from an assemblage of early successional forest species representing major functional guilds within a typical longleaf pine–wiregrass community: (1) a C$_3$ evergreen conifer (*Pinus palustris*); (2) a C$_4$ bunch grass (*Aristida stricta*); (3) a C$_3$ broadleaf tree (*Quercus margaretta*); (4) a C$_3$ perennial herbaceous legume (*Crotalaria rotundifolia*); and (5) a C$_3$ herbaceous perennial (*Asclepias tuberosa*). After 2 years, CO$_2$-enriched plots had 109% greater above-ground biomass than ambient plots, mainly due to a 117% increase in pine biomass. Community structure was altered by CO$_2$ enrichment; *Crotalaria* and *Asclepias* had higher mortality and less biomass in high-CO$_2$ plots, suggesting that not all species will perform well as global [CO$_2$] rises. Our data suggest that longleaf pine communities as a whole will perform well in a future higher CO$_2$ world, but some species may fall prey to altered competitive interactions for light and soil moisture.

Key-words: *Aristida stricta*, competition, elevated CO$_2$, *Pinus palustris*

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Introduction

Individual canopy and understorey forest species differ in morphology, physiology, life form, symbiotic relationships, and reproductive strategies, and these differences often correlate with differential responses to elevated CO$_2$ (Reekie & Bazzaz 1989; Drake 1992; Poorter 1993; Reich *et al*. 2001). Interspecific differences in resource acquisition efficiency, both above- and below-ground, may enhance or depress the CO$_2$ growth responses of individual community members, thereby affecting overall community response to elevated CO$_2$ (Reekie & Bazzaz 1989; Körner & Arnone 1992).

Leaf morphology (broadleaf vs. needle) and phenology (deciduous vs. evergreen) can affect growth responses to CO$_2$ enrichment, although there are discrepancies in the literature regarding comparative responses of conifers and broadleaf trees (Ceulemans & Mousseau 1994; Saxe *et al*. 1998; Pritchard *et al*. 1999). Leaf physiology also affects plant response, with plants utilizing a C$_3$ photosynthetic pathway, for example, being generally more responsive to increased atmospheric CO$_2$ than C$_4$ species (Drake 1992; Bowes 1993; Poorter 1993; Amthor 1995; Amthor & Loomis 1996). Symbiotic relationships with microorganisms may also be important, e.g. CO$_2$-induced increases in N$_2$-fixation may support the increased N demands precipitated by CO$_2$-enrichment (Hartwig *et al*. 1996). Consequently, CO$_2$-induced shifts in competitive advantages among species may alter species composition and community structure in mixed communities (Wray & Strain 1987; Fajer 1989; Joel *et al*. 2001).

To assess the performance of regenerating longleaf pine forests to future [CO$_2$], we constructed a model regenerating longleaf pine community composed of species representing different structural and functional

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guilds. We predicted differences in growth responses among plants differing in physiology, leaf structure and phenology, and symbiotic relationships. We predicted the following differences in growth responses: (1) broadleaf tree > conifer; (2) C$_3$ > C$_4$ plants; and (3) legumes > non-leguminous plants. These differences would cause shifts in species dominance and community structure, thus reducing the importance of longleaf pine and wiregrass. These results document two years of growth under both ambient and twice-ambient [CO$_2$] (720 μL CO$_2$ L^{-1}).

Materials and methods

STUDY SITE

We constructed a model regenerating longleaf pine–wiregrass ecosystem in spring 1998 at the National Soil Dynamics Laboratory in Auburn, Alabama, United States. The model forest community was assembled on an outdoor soil bin (2 m deep, 6 m wide, and 76 m long) containing a Blanton loamy sand (loamy, siliceous, thermic Grossarenic Paleudults) taken from a longleaf pine area (Sandhills or Subxeric Gulf Coastal Plain type, *sensu* Peet & Allard 1993) typical of the South-eastern Coastal Plains. Soil was collected in the Tuskegee National Forest in Macon County, Alabama. Soil had been in place for over 25 years, and had remained fallow for much of that time.

STUDY SPECIES AND COMMUNITY

Longleaf pine savannahs once occupied 37.2 million ha of the south-eastern United States (Landers *et al.* 1995). Following European settlement, timber harvesting, fire suppression, and conversion of forests to farmland reduced the land area of these ecosystems to less than 4% of their original range (Peet & Allard 1993; Landers *et al.* 1995).

These ecosystems have high diversity; some specific types have the highest reported values for species richness in the temperate Western Hemisphere (e.g. 140 species ha^{-1} for Mesic Longleaf Woodlands, Peet & Allard 1993). Xeric (Sandhills type) longleaf pine savannahs are dominated by longleaf pine, *Pinus palustris* Mill., but often have well-established mid-canopies consisting of xerophytic oak species (turkey oak, *Q. laevis* Walt., sand post oak, *Q. margaretta* Ashe, and bluejack oak, *Q. incana* Bart.) (Glitzenstein *et al.* 1995). The herbaceous layer is dominated by grasses, especially wiregrass, *Aristida stricta* Michx. (Abrahamson & Hartnett 1990), but the diverse legumes also present include annual herbs (*Chamaecrista fasciculata* [Michx] Greene), vines (*Clitoria mariana* L.), shrubs (*Amorpha fruticosa* L.), and perennial herbs (*Crotalaria rotundifolia* Walt. ex Gmel., *Tephrosia virginiana* [Walt.] Gmel.) (Hainds *et al.* 1999; Maceina *et al.* 2000).

We selected an assemblage of five early successional forest species to represent major functional guilds

within a typical longleaf pine–wiregrass community: *P. palustris* (a C$_3$ evergreen conifer); *Ar. stricta* (a C$_4$ bunch grass); *Q. margaretta* (a C$_3$ broadleaf tree); *C. rotundifolia* (a C$_3$ perennial herbaceous legume); and *Asclepias tuberosa* L. (a C$_3$, non-leguminous, herbaceous perennial). These species are common associates throughout the south-eastern U.S., and *P. palustris* and *Ar. stricta* are keystone species in the overstorey and understorey, respectively, of Sandhills longleaf pine savannahs (Abrahamson & Hartnett 1990). Prior to transplanting into the model community, all plants were grown in 15 cm^3 containers from seed collected from natural sources.

COMMUNITY DESIGN

Prior to planting, the soil bin was divided into 0.75 m^2 quadrats each possessing 16 equally spaced planting positions (see Pritchard *et al.* 2001). The community was constructed by randomly assigning individuals of each species (three *Pinus*, three *Aristida*, two *Quercus*, one *Crotalaria*, and one *Asclepias*) into assigned positions within each quadrat with six planting spaces per quadrat were left empty. This achieved target densities reflective of naturally regenerating Sandhills longleaf pine–wiregrass ecosystems (Hainds 1995; Jacqmain 1996). Open-top chambers encompassing 7.3 m^2 of ground surface area were used to deliver target [CO$_2$] of 365 μL L^{-1} (ambient) or 720 μL L^{-1} (elevated) beginning June 1998, using a delivery system described by Mitchell *et al.* (1995). The bin was divided into six blocks, and CO$_2$ treatments were randomly assigned to the chambers within each block.

Plants were regularly irrigated during summer 1998 to facilitate community establishment, using a metered drip irrigation system to deliver exact and consistent watering throughout the bin. During the first 2 months, any dead plants were replaced, but thereafter, mortality was attributed to causes other than transplanting, and gaps were not refilled. To mimic better the xeric nature of a Sandhills type longleaf pine forest, plants received only ambient rainfall once the community had established (August 1998) unless supplemental irrigation was necessary to prevent drought-induced mortality.

BIOMASS ESTIMATES

Estimates of total biomass in each chamber were made in autumn 1998, spring 1999, autumn 1999, and spring 2000. For estimates of initial above-ground biomass (dry mass) per plant, samples of seedlings from each species were destructively harvested from outside the chambers, 1 week after planting in spring 1998. For this and all subsequent estimations, the mean dry mass per plant per chamber for each species was multiplied by the number of individuals of each species within each chamber to estimate total above-ground biomass per chamber. To minimize disturbance within the chambers,

Table 1 Summary of estimation procedures used to derive above-ground biomass variables

Species	Date of data collection	Leaf dry wt. per plant	Stem dry wt. per plant	Total dry wt. per plant
Pinus	Spring 1998	A_{Pinus}	B_{Pinus}	$A_{pinus} + B_{Pinus}$
	Autumn 1998	$C_{Pinus} \times D_{Pinus}$	$E_{Pinus(I)}$	$(C_{Pinus} \times D_{Pinus}) + E_{Pinus(I)}$
	Spring 1999	$C_{Pinus} \times D_{Pinus}$	$F_{Pinus(I)}$	$(C_{Pinus} \times D_{Pinus}) + F_{Pinus(I)}$
	Autumn 1999	$C_{Pinus} \times D_{Pinus}$	$F_{Pinus(J)}$	$(C_{Pinus} \times D_{Pinus}) + F_{Pinus(J)}$
	Spring 2000	$L_{Pinus} \times D_{Pinus}$	$F_{Pinus(J)}$	$(L_{Pinus} \times D_{Pinus}) + F_{Pinus(J)}$
Quercus	Spring 1998	$A_{Quercus}$	$B_{Quercus}$	$A_{quercus} + B_{Quercus}$
	Autumn 1998	$C_{Quercus} \times D_{Quercus}$	$F_{Quercus(I)}$	$(C_{Quercus} \times D_{Quercus}) + F_{Quercus(I)}$
	Spring 1999	$C_{Quercus} \times D_{Quercus}$	$F_{Quercus(I)}$	$(C_{Quercus} \times D_{Quercus}) + F_{Quercus(I)}$
	Autumn 1999	$C_{Quercus} \times D_{Quercus}$	$F_{Quercus(J)}$	$(C_{Quercus} \times D_{Quercus}) + F_{Quercus(J)}$
	Spring 2000	$K_{Quercus} \times D_{Quercus}$	$F_{Quercus(J)}$	$(K_{Quercus} \times D_{Quercus}) + F_{Quercus(J)}$
Aristida	Spring 1998	$A_{Aristida}$	–	$A_{Aristida}$
	Autumn 1998	$C_{Aristida} \times D_{Aristida}$	–	$C_{Aristida} \times D_{Aristida}$
	Spring 1999	$C_{Aristida} \times D_{Aristida}$	–	$C_{Aristida} \times D_{Aristida}$
	Autumn 1999	$C_{Aristida} \times D_{Aristida}$	–	$C_{Aristida} \times D_{Aristida}$
	Spring 2000	$M_{Aristida} \times D_{Aristida}$	–	$M_{Aristida} \times D_{Aristida}$
Asclepias	Spring 1998	$A_{Asclepias}$	$B_{Asclepias}$	$A_{asclepias} + B_{Asclepias}$
	Autumn 1998	–	–	–
	Spring 1999	$C_{Asclepias} \times D_{Asclepias}$	$F_{Asclepias(I)}$	$(C_{Asclepias} \times D_{Asclepias}) + F_{Asclepias(I)}$
	Autumn 1999	–	–	–
	Spring 2000	$C_{Asclepias} \times D_{Asclepias}$	$F_{Asclepias(J)}$	$(C_{Asclepias} \times D_{Asclepias}) + F_{Asclepias(J)}$
Crotalaria	Spring 1998	$A_{Crotalaria}$	$B_{Crotalaria}$	$A_{crotalaria} + B_{Crotalaria}$
	Autumn 1998	$C_{Crotal.} \times D_{Crotal.}$	$N_{Crotal.} \times O_{Crotal.}$	$(C_{Crotal.} \times D_{Crotal.}) + (N_{Crotal.} \times O_{Crotal.})$
	Spring 1999	$C_{Crotal.} \times D_{Crotal.}$	$N_{Crotal.} \times O_{Crotal.}$	$(C_{Crotal.} \times D_{Crotal.}) + (N_{Crotal.} \times O_{Crotal.})$
	Autumn 1999	–	–	–
	Spring 2000	$C_{Crotal.} \times D_{Crotal.}$	$N_{Crotal.} \times O_{Crotal.}$	$(C_{Crotal.} \times D_{Crotal.}) + (N_{Crotal.} \times O_{Crotal.})$

A, mean leaf dry wt. per plant from destructively harvested seedlings at time of planting.
B, mean stem dry wt. from destructively harvested seedlings at time of planting.
C, mean number of leaves per plant ($n = 3$).
D, mean dry wt. per leaf from destructively harvested leaves within each chamber.
E, mean stem dry wt. from basal diameter regression.
F, mean stem dry wt. from height regression.
G, mean basal diameter ($n = 3$).
H, mean plant or stem ht ($n = 3$).
I, mean basal diameter of stem or clump (n = all individuals of species per chamber).
J, mean plant or stem ht (n = all individuals of species per chamber).
K, mean number of leaves per plant (n = all individuals of species per chamber).
L, mean number of leaves per plant from height regression.
M, mean number of leaves per plant from basal diameter regression.
N, mean number of runners per plant ($n = 3$).
O, mean dry wt. of stem per runner from destructively harvested runners within each chamber.
Regression parameters are in text and Table 2.

biomass estimates in autumn 1998 and spring 1999 were made from non-destructive measurements taken on three plants of each species within each chamber, arbitrarily selected to represent the range of sizes present. In autumn 1999 and spring 2000, the community was well established, and all plants in each chamber were used to obtain biomass estimates. Specific estimation procedures for each species were as follows (see Table 1 for brief synopsis).

Pinus palustris

In autumn 1998, spring 1999 and autumn 1999, all needles were counted on each of three selected pines to obtain the mean number of needles per pine for each chamber. In spring 2000, an allometric equation was used to estimate the number of needles per plant from stem height (Table 2). For stem dry mass estimations, allometric equations were generated from data on pines collected from natural populations (see Table 2). The growth pattern of longleaf pine saplings consists of a 'grass' stage when stems increase in girth but not height, followed by bolting, after which vertical growth is favoured: different variables were therefore used for dry mass regressions, depending on the growth stage of the saplings. In autumn 1998, estimates of stem dry mass were generated from a basal diameter regression (Newton pines). In spring 1999, stem height and basal diameter were used to generate separate regressions for a different sample of pines ($r^2 = 0.98$ and $r^2 = 0.67$, respectively), and the better estimate (stem height) was used in all subsequent estimations.

Table 2 Allometric equations for estimating biomass

Species	Dependent variable (y)	Independent variable (x)	Regression equation	R^2	P-value	n	Date(s) used
Pinus[a]	Stem dry wt.	Basal diameter	$y = (-6.45) + (1.09x)$	0.73	< 0.0001	72	Autumn 1998, spring 1999
Pinus[b]	Stem dry wt.	Stem height	$y = (-19.48) + (1.53x)$	0.98	< 0.0001	8	Autumn 1999, spring 2000
Pinus[b]	Needle number	Stem height	$y = (695) + (26x)$	0.92	0.0199	10	Spring 2000
Quercus[b]	Stem dry wt.	Stem height	$y = (-0.80) + (0.12x)$	0.72	0.0158	7	Autumn 1998, spring, autumn 1999, spring 2000
Quercus[b]	Branch dry wt.	Stem height	$y = (-0.09) + (0.02x)$	0.92	< 0.0001	10	Autumn 1999, spring 2000
Aristida[b]	Tiller number	Clump diameter	$y = (-191) + (17x)$	0.95	< 0.0001	10	Spring 2000
Asclepias[c]	Stem dry wt.	Basal diameter	$y = (-1.75) + (0.96x)$	0.77	< 0.0001	24	Spring 1999, spring 2000

Lower case superscripts denote source of destructively harvested plants: a, Jones Ecological Center, Newton, GA; b, non-experimental plots within the model community, Auburn, AL; c, Tuskegee National Forest, Tuskegee, AL.

We recognize the problems inherent in using allometric equations to estimate biomass (see Norby *et al.* 2001), but we were unable to destructively harvest experimental plants without compromising community structure. Like Norby *et al.* (2001), however, our allometric equations were robust and our sampling error was minimal (every plant was measured after spring 1999), thus we are confident in our estimates.

Quercus margaretta

The mean number of leaves per plant per chamber was estimated by counting all leaves on three oaks within each chamber for autumn 1998, spring 1999, and autumn 1999. In spring 2000, leaves were counted on all oaks. Stem dry mass was estimated from mean stem heights (Table 2) using three plants per chamber in autumn 1998 and spring 2000, and all oak stems thereafter. Branches did not comprise a significant amount of oak biomass until autumn 1999 and their dry mass was obtained from an allometric equation (Table 2).

Aristida stricta

Separate estimations of stem and leaf biomass were not made for wiregrass; instead, the total number of tillers per plant on three selected plants within each chamber was counted in autumn 1998, spring 1999 and autumn 1999. In spring 2000, plants were harvested from non-experimental plots within the model community to create an allometric equation for estimating the number of tillers per plant from plant diameter (Table 2).

Crotalaria rotundifolia

The procumbent growth form of *Crotalaria* made biomass estimations difficult and it was therefore critical that similar procedures were used throughout. For each collection date, three previously unmeasured plants were selected within each chamber, and a plexiglass triangle (45° ×67.5° ×67.5°) was placed horizontally on top to delineate one-eighth of the plant area, assumed to be circular, with the 45° angle at the centre. The left side of the triangle (viewed from the centre of the plant) was lined up with the same compass aspect within each chamber, using a randomly selected aspect for each season. The total number of runners within the boundaries of the triangle was counted and three representative runners were destructively harvested and multiplied by eight to obtain biomass estimates per plant per chamber. Although evergreen throughout much of its range, *Crotalaria rotundifolia* leaf senescence can occur when plants are stressed (drought or frost) in autumn. This was the case in autumn 1999, therefore no attempt was made to estimate biomass for that period.

Asclepias tuberosa

For spring 1999 and 2000, an allometric equation was created for estimating stem biomass from stem basal diameter (Table 2), and leaves were counted on all plants within each chamber. *Asclepias tuberosa* senesces in late summer and overwinters as a tuber, therefore no above-ground biomass estimates were obtained for autumn 1998 and 1999.

STATISTICAL ANALYSIS

Data were analysed with one-way analyses of variance (ANOVAS) taking into account the randomized block design. To meet better the assumptions underlying ANOVA, all count data were square root transformed and all percentages were arcsine-square root transformed prior to analysis (Zar 1996). STATVIEW 5.0 was used for all analyses (SAS 1998). Differences were considered significant at α ≤0.05 and trends were recognized at 0.05 ≤ α ≤0.15.

Results

SPECIES RESPONSES

Pinus palustris

Total above-ground biomass of *Pinus* was greater in CO$_2$-enriched plots for all data collection periods (Fig. 1) by 53%, 21%, 39%, and 117% for autumn 1998,
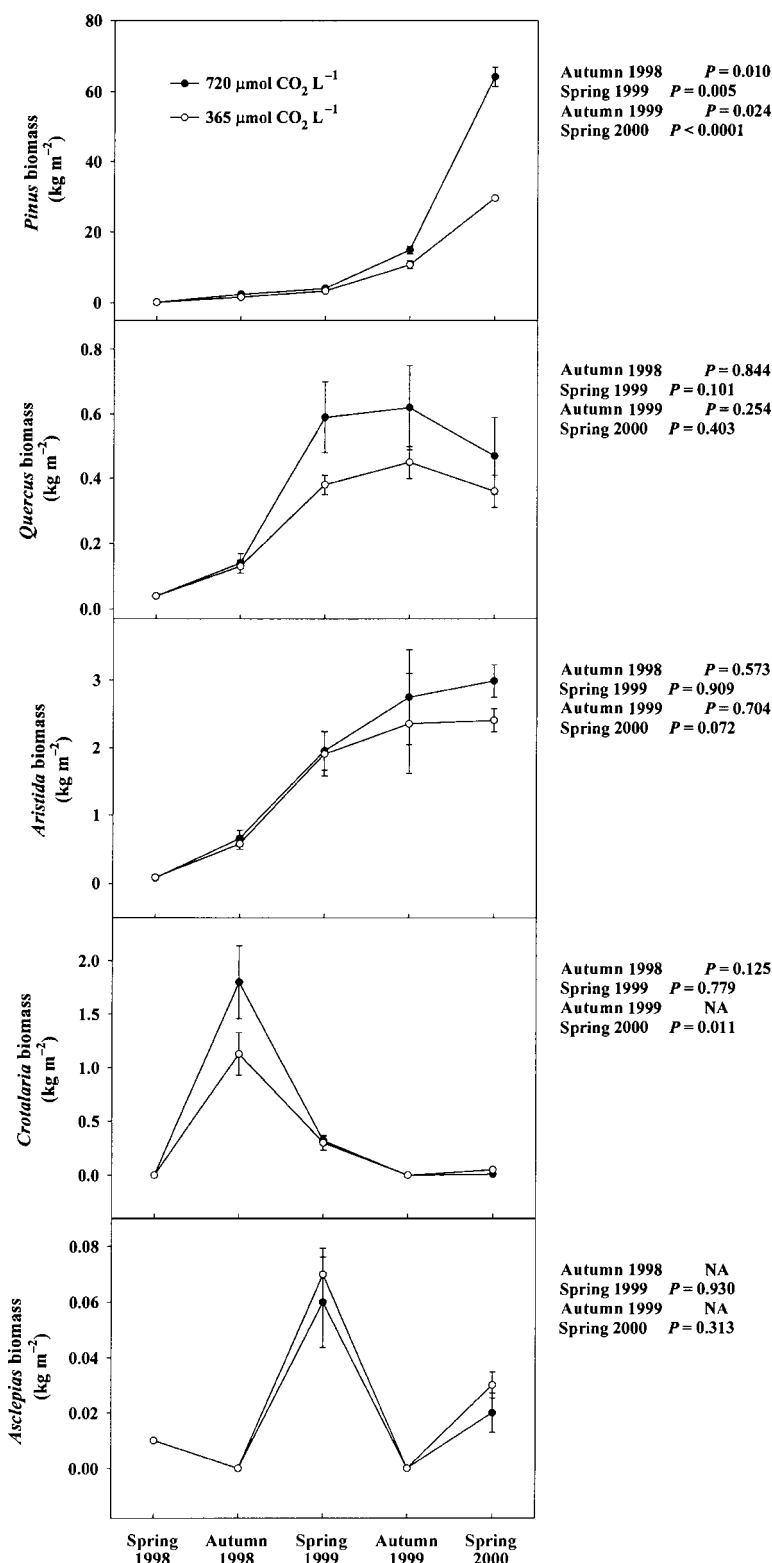
Fig. 1 Total above-ground biomass of each species from spring 1998 to spring 2000. Bars represent ±1 SE.

spring 1999, autumn 1999, and spring 2000, respectively. During the first growing season, the treatment difference was due to increased leaf dry mass per plant (Table 3). By autumn 1999, plants had begun to bolt from the grass stage, and increases in both stem and leaf mass contributed to subsequent increases in overall biomass. By spring 2000, above-ground dry mass per plant was 90% greater, and plants were 30 cm taller (both $P < 0.0001$) in elevated compared to ambient plots. Longleaf pine can experience multiple growth flushes in a single growing season; here, CO_2-enriched trees had an extra growth flush in 1999 and 2000 (Table 3, $P = 0.003$ and $P < 0.0001$, respectively). Significant branching was not observed until spring 2000, but

Table 3 Responses of above-ground biomass variables to elevated CO$_2$

Season	Biomass variable	Pinus	Quercus	Aristida	Crotalaria	Asclepias
Autumn 1998	Above-ground dry wt. (g)	+37%*	NR	NR	+71%*	–
	Leaf area per plant (cm^2)	+53%**	NR	NR	+48%^{tr}	–
	Leaf dry wt. per plant (g)	+54%**	NR	–	+67%*	–
	Stem dry wt. (g)‡	NR	NR	–	+72%^{tr}	–
	Plant ht (cm)	–	NR	+28%^{tr}	–	–
	Basal diameter (cm)†	NR	NR	NR	NR	–
	Number of leaves	NR	NR	NR	+62%^{tr}	–
	Dry wt. per leaf (g)	+27%*	NR	NR	NR	–
	Area per leaf (cm^2)	+26%*	NR	NR	NR	–
	Specific leaf area (cm^2 g^{-1})	NR	NR	NR	NR	–
	Number of branches	–	NR	–	–	–
	Number of flushes	–	–	–	–	–
	Branch dry wt. (g)	–	NR	–	–	–
	Total branch length (cm)	–	NR	–	–	–
	Number of stems§	–	–	–	NR	–
Spring 1999	Above-ground dry wt. (g)	+10%***	NR	NR	NR	NR
	Leaf area per plant (cm^2)	NR	NR	NR	NR	NR
	Leaf dry wt. per plant (g)	+17%^{tr}	NR	–	NR	NR
	Stem dry wt. (g)‡	NR	NR	–	NR	NR
	Plant ht (cm)	NR	NR	NR	–	NR
	Basal diameter (cm)†	NR	–	NR	–	NR
	Number of leaves†‡	NR	NR	NR	NR	NR
	Dry wt. per leaf (g)	NR	NR	NR	NR	NR
	Area per leaf (cm^2)	NR	NR	NR	NR	NR
	Specific leaf area (cm^2 g^{-1})	NR	NR	NR	NR	–20%**
	Number of branches†	–	NR	–	–	–
	Number of flushes†	–	–	–	–	–
	Branch dry wt. (g)	–	NR	–	–	–
	Total branch length (cm)	–	NR	–	–	NR
	Number of stems§	–	–	–	NR	NR
Autumn 1999	Above-ground dry wt. (g)	+24%^{tr}	NR	NR	–	–
	Leaf area per plant (cm^2)	+26%^{tr}	NR	NR	–	–
	Leaf dry wt. per plant (g)	NR	NR	–	–	–
	Stem dry wt. (g)‡	+25%***	NR	–	–	–
	Plant ht (cm)	+18%***	NR	NR	–	–
	Basal diameter (cm)†	+3%*	+13%*	NR	–	–
	Number of leaves	+25%^{tr}	NR	NR	–	–
	Dry wt. per leaf (g)	NR	NR	NR	–	–
	Area per leaf (cm^2)	NR	NR	NR	–	–
	Specific leaf area (cm^2 g^{-1})	NR	NR	NR	–	–
	Number of branches	NR	NR	–	–	–
	Number of flushes	+20%**	–	–	–	–
	Branch dry wt. (g)	–	NR	–	–	–
	Total branch length (cm)	–	NR	–	–	–
	Number of stems§	–	–	–	–	–
Spring 2000	Above-ground dry wt. (g)	+90%***	+27%^{tr}	+25%***	–59%^{tr}	+44%*
	Leaf area per plant (cm^2)	+73%***	NR	+28%***	–64%*	+70%*
	Leaf dry wt. per plant (g)	+92%***	+29%^{tr}	–	–64%*	+74%*
	Stem dry wt. (g)‡	+72%***	+15%^{tr}	–	NR	NR
	Plant ht (cm)	+55%***	+12%^{tr}	+15%***	–	NR
	Basal diameter (cm)†	+6%**	NR	+8%**	NR	NR
	Number of leaves	*	NR	+ 11%**	–57%*	NR
	Dry wt. per leaf (g)	+21%*	NR	+14%^{tr}	–20%^{tr}	NR
	Area per leaf (cm^2)	NR	NR	NR	NR	NR
	Specific leaf area (cm^2 g^{-1})	–10%*	NR	NR	+13%^{tr}	NR
	Number of branches	+219%***	NR	–	–29%^{tr}	–
	Number of flushes	+23%***	–	–	–	–
	Branch dry wt. (g)	–	NR	–	–	–
	Total branch length (cm)	–	NR	–	–52%*	NR
	Number of stems§	–	–	–	–35%*	NR

†Stem diameters for *Pinus*, *Quercus*, and *Asclepias*; clump diameters for *Aristida* and *Crotalaria*.
‡Whole tillers were counted as leaves for *Aristida*, hence no stem data were presented; *Aristida* above-ground dry wt. is total tiller ('leaf') dry wt.
§Runners were considered stems for *Crotalaria*.
*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, $^{tr}0.15 > P > 0.05$, NR = no response.

treatment differences were then dramatic. (+219% branches per tree and almost 50% vs. 18% of trees having branches, both $P < 0.0001$). Mortality did occur (17% of CO_2-enriched pines and 19% of ambient pines died) but did not differ between CO_2 treatments.

Quercus margaretta

No significant differences were detected in total above-ground oak biomass, although in spring 1999 it was 55% greater in plots exposed to elevated CO_2 (Fig. 1). In general, morphological characteristics did not differ between treatments until spring 2000 when both leaves (+29%, $P = 0.109$) and stems (+15%, $P = 0.080$) contributed to a 27% increase in above-ground biomass per plant ($P = 0.109$). Extensive leaf measurements in autumn 1999 showed that leaves grown in elevated CO_2 did not differ from those grown under ambient conditions for leaf area, length, width, and perimeter (data not shown). Total oak biomass for both CO_2 treatments was reduced in spring 2000 compared to autumn 1999. Individual oak plants in ambient chambers had a 9% mean increase in biomass from autumn 1999 to spring 2000, but suffered 15% cumulative mortality. Oak plants exposed to elevated CO_2 suffered 13% cumulative mortality, but also had a 39% reduction in biomass per plant due mainly to stem and branch abscission. There was no significant difference in mortality between CO_2-enriched and ambient-grown oaks.

Aristida stricta

Wiregrass exhibited little response to elevated CO_2 until spring 2000 (Table 3). At that time, CO_2-enriched plots had 24% more total wiregrass biomass than ambient plots ($P = 0.072$), due to an 11% increase in the number of tillers ($P = 0.008$) and a 14% increase in dry mass per tiller ($P = 0.104$). CO_2-fertilized plants were also 15% taller ($P < 0.0001$). Individual tillers exhibited no CO_2 response for dry mass, area, or specific leaf area, nor did mortality differ between treatments. Overall, wiregrass growth in ambient chambers levelled off by spring 2000. Among all species, cumulative mortality was least for wiregrass and did not significantly differ between treatments (5% mortality for both CO_2 treatments).

Crotalaria rotundifolia

Above-ground *Crotalaria* biomass per chamber peaked for both CO_2 treatments in autumn 1998, and then dramatically declined (Fig. 1). Likewise, the benefit realized from exposure to elevated CO_2 peaked in autumn 1998, disappeared in spring 1999, and finally became a detriment in spring 2000 (Table 3, Fig. 1). In autumn 1998, above-ground biomass of plants in high CO_2 was 71% greater than that of plants under ambient CO_2 ($P = 0.044$), however, ground cover per plant did not differ between CO_2 treatments (data not shown). In

spring 1999, no CO_2 response was detected for any *Crotalaria* variables, and early senescence prevented measurement of *Crotalaria* plants in autumn 1999. In spring 2000, biomass was greater in ambient than CO_2-enriched plots. Plants grown in chambers exposed to elevated CO_2 had 59% less above-ground biomass ($P = 0.096$), 64% less leaf area per plant ($P = 0.044$), 64% less leaf dry mass per plant ($P = 0.047$), and 57% fewer leaves ($P = 0.019$) compared to plants in ambient CO_2 chambers. Morphologically, *Crotalaria* plants exposed to 2 years of elevated CO_2 had 35% fewer stems (runners) ($P = 0.029$), 29% fewer branches ($P = 0.089$), and 52% less total stem length ($P = 0.024$) than plants exposed to ambient CO_2 (spring 2000 data, Table 3).

Although mortality played a significant role in chamber-level reduction of *Crotalaria* biomass (see below), biomass per plant was dramatically smaller for both CO_2 treatments in spring 2000. Biomass per plant of ambient-grown *Crotalaria* plants in spring 1999 was 25% that at its peak (autumn 1998); by spring 2000, it was only 7%. The reduction in plant size was even more dramatic in CO_2-enriched chambers; for spring 1999 and spring 2000, biomass per plant was only 17% and 2% that of autumn 1998 peak biomass per plant, respectively. Cumulative *Crotalaria* mortality was high for both CO_2 treatments, but was higher for CO_2-enriched chambers than for ambient chambers (52% and 28%, respectively; $P = 0.067$; Fig. 2).

Asclepias tuberosa

Individual *Asclepias* plants varied greatly in size (e.g. plant height ranged from 4 to 87 cm in spring 2000), thus differences in above-ground biomass were difficult to detect. Nevertheless, in spring 2000, plants exposed to elevated CO_2 realized a 44% increase in above-ground biomass ($P = 0.045$), a 74% increase in leaf dry mass per plant ($P = 0.015$), and a 70% greater leaf area per plant ($P = 0.015$) relative to ambient-grown plants. Although biomass per plant in spring 2000 was greater in plots exposed to elevated CO_2, there was 49% greater mortality in CO_2-enriched plots compared to ambient
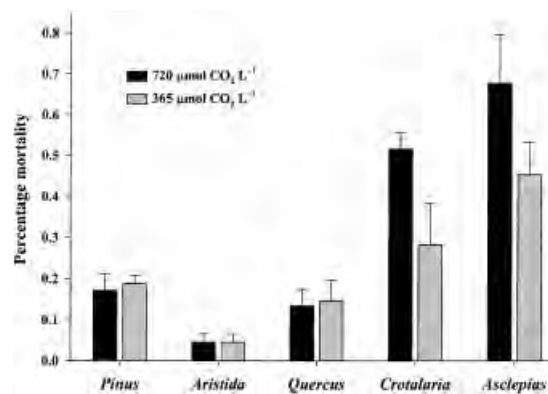

Fig. 2 Percentage mortality of each species (no. of plants in spring 1998 – no. of plants in spring 2000/no. of plants in spring 2000). Bars represent ±1 SE.
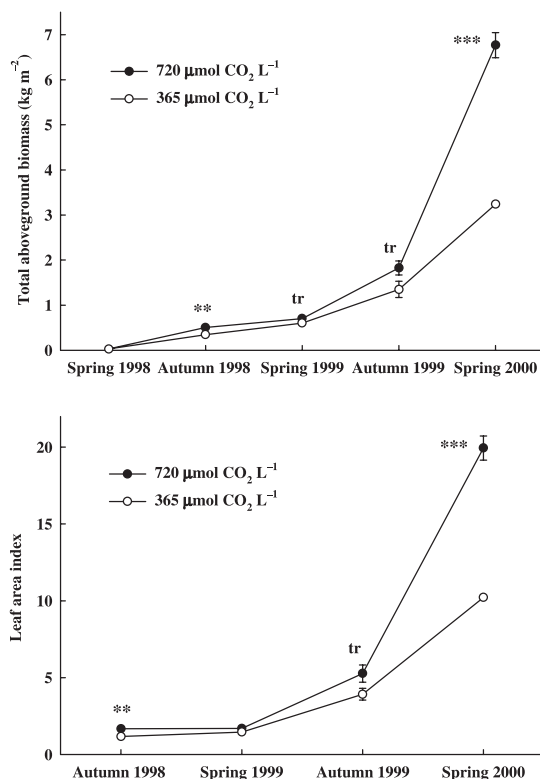
Fig. 3 Total above-ground biomass (kg m^{-2}) of all species combined and leaf area index. Note that LAI includes total leaf of all species area per m^2. Bars represent ±1 SE. *$P < 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$, $^{tr}0.15 \geq P \geq 0.05$.

Fig. 4 Percentage of total above-ground biomass comprised by each species from autumn 1998 to spring 2000.

plots ($P = 0.131$; Fig. 2). Thus, no response to CO_2 enrichment was detected for total above-ground biomass per chamber in spring 2000. Similar to *Crotalaria*, *Asclepias* biomass per chamber peaked and then declined in both ambient and elevated CO_2 plots. From spring 1999 to spring 2000, biomass per plant was reduced by 72% and 58% for CO_2-enriched and ambient-grown plants, respectively.

COMMUNITY LEVEL RESPONSES

The total above-ground biomass of CO_2-enriched plots was greater than that of ambient plots at all assessment dates; significantly so in autumn 1998 and spring 2000 when it was 109% greater (Fig. 3). Leaf area index (LAI, total leaf area per m^2) was greater in elevated CO_2 chambers for most assessment dates (Fig. 3). LAI values may appear high, but, all leaf area was included in these estimates, including herbaceous understorey species, and vertical distribution of needles on stems of young longleaf pine saplings also leads to greater leaf area per m^2 than in a mature forest. The greatest difference in LAI was again seen in spring 2000 when it was 95% greater in elevated CO_2 plots.

Changes in community structure with respect to time were observed (e.g. the percentage of total biomass comprised by *Pinus* increased at each assessment date). Contributions by individual species to total above-ground biomass did not, however, differ between CO_2
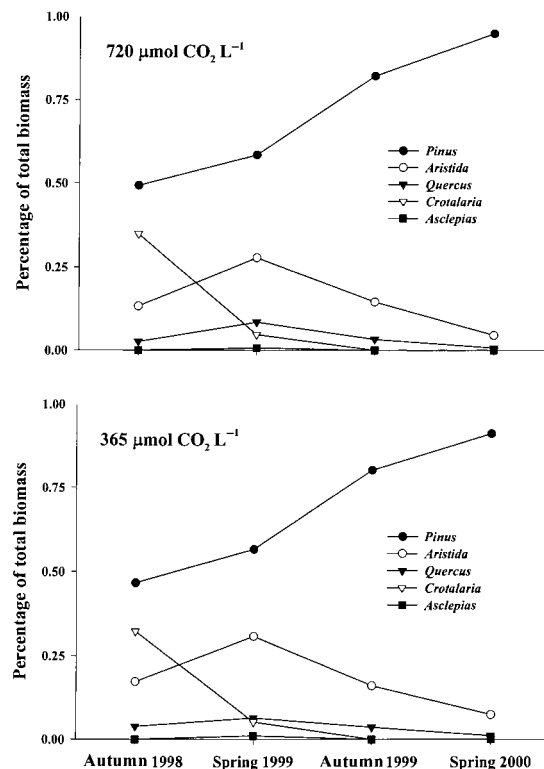
treatments until spring 2000, however, when the doubled *Pinus* biomass in the CO_2-enriched treatment led to significant differences in community composition (95% vs. 91% of total biomass was *Pinus*, $P = 0.0003$, 5% vs. 7% was *Aristida*, $P = 0.0004$, Fig. 4). *Quercus* biomass comprised slightly more of the total weight of ambient CO_2 plots (1.1% vs. 0.7%, $P = 0.088$), but values for *Crotalaria* and *Asclepias* were lowest under elevated CO_2 (0.02% vs. 0.2%; $P = 0.0003$; 0.03% vs. 0.1%; $P = 0.014$, respectively).

Discussion

The responses of individual plant species to elevated CO_2 vary, but, generally, most plants experience an increase in photosynthesis and growth when exposed to enhanced atmospheric CO_2. Unfortunately, data that describe how intact plant communities will respond to a high CO_2 world are sparse, especially for dry upland communities such as savannahs and scrub forests (Arnone 1996). We tested the response of a community modelling a regenerating Sandhills-type longleaf pine savannah to relatively long-term (2-year) exposure to elevated atmospheric CO_2. The overall community response was positive with 109% greater biomass (Fig. 3), but as predicted, individual species responded differently although with unexpected patterns.

We predicted that *Q. margaretta* would have a greater biomass response to CO_2 enrichment than the slow-growing *P. palustris*. However, at every assessment

Pinus plants had greater above-ground biomass when CO_2-enriched, whereas the C_3 broadleaf *Q. margaretta* never exhibited a statistically significant effect of CO_2 for any growth parameter (Table 3). At both the whole plant and community levels, *P. palustris* realized the greatest benefit, with 117% greater above-ground biomass and 4% more of the total community biomass in CO_2-enriched than in ambient plots. Individual *Pinus* plants grown under elevated CO_2 were taller, had greater stem mass, produced more leaves, and had more branches (Table 3, Fig. 1).

Total above-ground oak biomass per chamber was reduced in both treatments in spring 2000 (Fig. 1). Responses of individual oaks were however, extremely variable with final heights ranging from 12 cm to 167 cm, but the mean (< 40 cm) was 30 cm less than the value for pine. This, coupled with the greater LAI in CO_2-enriched plots may have increased competition for light and thus inhibited the growth response of many oaks. Many of the smaller oaks were in dense shade (from pines) and experienced significant stem and branch abscission.

Exposure to elevated CO_2 often increases photosynthetic rates. In this model community, *Quercus* realized a 46% increase in photosynthesis in spring 1999 compared with 19% for *Pinus* (unpublished data), despite individual pines already having 10% more biomass in CO_2-enriched chambers. Root data from the model community showed that *Pinus* also realized a greater below-ground CO_2 benefit than *Quercus* (Pritchard *et al*. 2001). Clearly, the magnitude of photosynthetic responses to elevated CO_2 can be misleading when trying to predict subsequent growth responses. In our model community, mild winters and evergreen leaves allowed C fixation to continue year round for *P. palustris* (data not shown), whereas the deciduous nature of *Q. margaretta* limited C fixation, and hence the ability to benefit from CO_2 enrichment, to spring and summer months. Pritchard *et al*. (2001) suggested that the discrepancies in the literature regarding comparative responses of conifers and deciduous trees to CO_2 may be explained by considering northern pines and southern pines separately. Many northern species experience only one growth flush per year or only flush during spring, whereas southern pines (e.g. *P. palustris*, *P. taeda* L., and *P. elliottii* Engelm.) may experience multiple flushes throughout the entire growing season (Kozlowski & Pallardy 1997) and thus realize a greater benefit from exposure to elevated CO_2.

The anatomical and biochemical pathways by which atmospheric C enters a leaf cause photosynthesis, and thus biomass accumulation, in C_4 plants to be relatively insensitive to increases in CO_2 (Amthor & Loomis 1996). Here, however, *Ar. stricta*, the C_4 bunch grass, had 25% more above-ground biomass in plots exposed to CO_2 than ambient plots, albeit only after 2 years, with only *Pinus* realizing a greater benefit. This emphasizes the importance of long-term (> 1 year) experiments when assessing plant responses to CO_2. The evolution of the C_4 photosynthetic pathway is typically thought to have been an adaptation to warm, dry, high-light regions (Sage *et al*. 1999). Much of central and south Alabama experienced severe drought conditions during 2000. Mean maximum monthly temperatures were above 30 °C for eight of the 25 months of the study and above 25 °C for 13 months. Thus, species well-adapted for hot, dry conditions (i.e. *Aristida* and *Pinus*) had the greatest growth response to elevated atmospheric CO_2 in our experiment, where restricted watering maintained xeric conditions.

The availability of soil resources is predicted to limit long-term forest response to elevated CO_2 (Díaz *et al*. 1993; Luo & Mooney 1996; Tingey *et al*. 2000; Oren *et al*. 2001). Plant species that are able to capture soil resources more efficiently, via either root function or symbiotic interactions, are predicted to perform well in a future higher CO_2 world (Hartwig *et al*. 1996). Although at the first assessment date (autumn 1998) the N-fixing legume, *C. rotundifolia*, had the largest CO_2-induced biomass response per plant of all species (+71% total dry mass) (Table 3), by autumn 1999, its responses did not differ between treatments and CO_2-enriched plants actually had less above-ground biomass and greater mortality at the final date (Table 3, Fig. 2). The non-leguminous forb, *As. tuberosa*, had similarly reduced total biomass and greater mortality in CO_2-enriched plots in spring 2000 (Figs 1 and 2).

Nitrogen was not the only limiting resource in this experiment. Soil moisture was scarce for many of the summer months, and light was also limiting for some species. The root structure of *C. rotundifolia*, a central taproot with few lateral roots (personal observation, Duncan & Duncan 1999) makes it a poor competitor for soil moisture, and its procumbent growth habit makes it a poor competitor for light. By spring 2000, LAI was 95% greater in CO_2-enriched chambers, reducing the light levels near the soil.

Plots exposed to elevated CO_2 always had greater total biomass (Fig. 3). The percentage of each species' contribution to total biomass, however, did not differ between treatments until spring 2000 (Fig. 4), when a 4% increase in the contribution of *P. palustris* in CO_2-enriched plots than ambient plots was balanced by decrease in all other species. Although statistically significant, this shift in community structure would probably not alter community function, since both dominants, longleaf pine and wiregrass, performed well under CO_2 enrichment. A more distinctive response is greater mortality in CO_2-enriched plots for the forbs, *C. rotundifolia* and *As. tuberosa* (Fig. 2). Individual *Crotalaria* plants were also smaller in high CO_2 plots. Surviving *Asclepias* plants were larger in plots exposed to elevated CO_2, but all had perished in one-third of high CO_2 plots. Again, increased competition for light and soil moisture was the most likely cause for these differences in mortality. Longleaf forests depend on frequent fires to maintain biodiversity (Christensen 1987; Glitzenstein *et al*. 1995). Long-term exposure of

ecosystems to elevated atmospheric CO_2 may increase fire disturbance by increasing fuel load (as with the doubling of biomass seen here) and altering leaf chemistry (Sage 1996). Furthermore, the greatest increase in growth was realized by the two most pyrogenic species (*P. palustris* and *Ar. stricta*). Such increases in fuel load and rate of fuel accumulation could increase the likelihood and frequency of fire in natural longleaf ecosystems, and these effects of elevated atmospheric CO_2 need to be addressed in future studies.

Conclusions

Barring further anthropogenic degradation, Sandhills-type longleaf forests should perform well if CO_2 concentrations rise, with *P. palustris* and *Ar. stricta* remaining the keystone species. The pyric characteristics of this fire-dependent ecosystem should also be well-maintained. Caution should be used, however, when making further ecosystem-scale interpretations from this experiment. In our model community, two particular C_3 forbs performed poorly after long-term exposure to elevated CO_2, but frequently burned Sandhills savannahs typically have > 40 species ha^{-1} (Walker 1997) that represent a variety of growth forms, and it is therefore impossible to make accurate predictions for all or most species. However, longleaf ecosystems harbour a large number of threatened and endangered species (Walker 1997) and their current tenuous position in these communities may become more imperilled if these species cannot maintain or improve their competitive abilities as atmospheric [CO_2] continues to rise.

Acknowledgements

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